THE UNIVERSITY OF WISCONSIN
COLLEGE OF AGRICULTURE

Madison 6

DEPARTMENT OF GENETICS

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Dear Evelyn:

I've been owing you a letter for some time, I suppose- but not with any intentions of mystery-- the work simply hadn't gone far enough.

Lately, I've worked out reasonable methods for getting suspensions of diploid stocks which will contain only about 5% segregated cells. One diploid has been particularly useful, being doubly heterozygous in repulsion for Lac_1 and Lac4, closely linked. Consequently, to all intents and purposes, all Lac+ are diploid, while Lac- are haploid.

As you will have guessed by now, I've been treating diploid suspensions with radiations and chemicals to see how they work. Unfortunately, the X-ray facilities here are limited, and I haven't been able to do much detailed quantitative work. But it is quite clear that the UV survival curves of (1n) K-12, and of 2n diploids are superimposable. As Atwood finds in Neurospora, recessive lethals (detectable via balanced lethal, i.e., stabilized, diploids) play an unimportant role in killing. But radiations and a variety of chemicals have one very dramatic genetic correlate to sterilization: destruction or removal of entire chromosomes as detected by haploidizations. It is very simple to rule out selection, as with small, barely lethal doses of UV (pS ca. .2-.4) an appreciable fraction of the surviving diploids are now haploid, giving pure Lac- colonies (and usually also pure for other heterozygous factors, so the elimination is not restricted to this locus.) Chromosome removal is quite credible, as there are any number of descriptions of such losses in higher plants, although often categorized as "physiological" effects.

On the basis that organic peroxides and mustards have in common the property of being alkylating agents (capable of releasing highly reactive free radicals or C ions), a number of other compounds have been tested, and each of these has the same striking haploidising effect as UV or X-rays. Some of these reagents are quite unstable in aqueous solution, but can be handled with short time treatments, ca. 10 minutes. They include: N-mustard (HN2); formaldehyde ; acetic anhydride; benzoyl chloride; phenyl isocyanate; dimethyl sulfate. Some others are in the mill; one might mention that diazomethane is reported mutagenic in Neurospora. All of these compounds react freely with NH2; SH; ØOH, imidazole, etc., You can see why I needed some nonmutagenic chemicals for comparison! Heat Methyl Green; and Urethan are the only reagents so far tested which kill clearly without concomitant haploidization. Pyronin and Acriflavine appear to give the same result, but they clump the bacteria so heavily that it is difficult to evaluate the killing- or be sure that there is very much! I couldn't get enough

killing with desoxycholate to evaluate it in this system. Similarly with diphenyl iodonium chloride, reputedly a rather specific SH reagent.

Experience with Acriflavine and Pyronin makes me wonder how it is possible to verify the mutagenic effects reported. Since they agglutinate so heavily, they would appear to be mutafacient. Every clump, although it might contain very many sensitive bacteria, would be scored as a single, resistant survivor if it contained one resistant. Thus the apparent proportion of resistants would be augmented. This can be, and probably has been, checked with made-up reconstruction experiments. But the clumping could also interfere with the access of the phage to the bacteria- a caution you have also enunciated.

Urethan may be one of the most interesting chemical mutagens, if its mutagenic activity, on one hand, and its lack of haploidising effect, on the other, can be confirmed. Can you dig up the most critical information on this compound? I understand someone at CSH -- was it you or Bryson-- has been working with it, as well as Latarjet. The paradox may not be a real one: there might simply be a cellular mechanism of killing which obscures the haploidization. At any rate, this mightbe a way of tracking down some more specificor more subtle mutagens than the gross reagents we now have.

This stuff isn't quite ready for publication yet, so this doesn't belong in MGB just yet.

If you care for any experimental details about any compound for possible test as a mutagen, I'll be glad to send it.

Sincerely,

Joshua

Joshua Lederberg